Bioconversion of Wheat Straw to Ethanol: Chemical Modification, Enzymatic Hydrolysis, and Fermentation*

R. W. DETROY, L. A. LINDENFELSER, S. SOMMER, and W. L. ORTON, *Northern Regional Research Center, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Peoria, Illinois 61604*

Summary

Native wheat straw (WS) was pretreated with various concentrations of H_2SO_4 and NaOH followed by secondary treatments with ethylene diamine (EDA) and NH_4OH prior to enzymatic saccharification. Conversion of the cellulosic component to sugar varied with the chemical modification steps. Treatment solely with alkali yielded 51–75% conversions, depending on temperature. Acid treatment at elevated temperatures showed a substantial decrease in the hemicellulose component, whereas EDA-treated WS (acid pretreated) showed a 69–75% decrease in the lignin component. Acid-pretreated EDA-treated straw yielded a 98% conversion rate, followed by 83% for alkali–NH_4OH treated straws. In other experiments, WS was pretreated with varying concentrations of H_2SO_4 or NaOH followed by NH_4OH treatment prior to enzymatic hydrolysis. Pretreatment of straw with 2% NaOH for 4 h coupled to enzymatic hydrolysis yielded a 76% conversion of the cellulosic component. Acid–base combination pretreatments yielded only 43% conversions. A reactor column was subsequently used to measure modification–saccharification–fermentation for wheat straw conversion on a larger scale. Thirty percent conversions of wheat straw cellulosics to sugar were observed with subsequent fermentation to alcohol. The crude cellulase preparation yielded considerable quantities of xylose in addition to the glucose. Saccharified materials were fermented directly with actively proliferating yeast cells without concentration of the sugars.

INTRODUCTION

Current research in the development of alternative fuels has led to investigation of a variety of raw materials, including agricultural residues, from which ethanol might be synthesized. An estimated 400 million dry tons of these materials are produced annually, of which 70–85 million tons might be harvested without materially affecting soil tilth.[1]

* The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Biotechnology and Bioengineering, Vol. XXIII, Pp. 1527–1535 (1981)
Published by John Wiley & Sons, Inc.
 CCC 0006-3592/81/071527-09$01.00

The lignocellulose matrix of plant materials has been the subject of considerable study primarily for releasing bound cellulose from the lignin component. Studies have included the release of cellulose by both biological[2–4] and chemical processes,[5–17] and combinations of the two treatment methods.[2,3] The objectives included upgrading agricultural residues for use as farm ruminant feed,[5–10,11,13] and liberation of cellulose from plant materials for subsequent conversion to fermentable sugars.[18,19]

The economical production of fermentable sugars requires that essentially quantitative conversion of the hemicellulose and cellulose components be attained. In the treatment of cellulosic residues with alkali, the fiber structure is believed to swell, and the water-holding capacity and penetrability by microorganisms and enzymes are enhanced.[20] Hemicellulose, a polymer of primarily pentoses with some hexoses and sugar acids, can be converted rather easily to monomeric sugars by dilute H_2SO_4 at elevated temperatures.[21] An excellent review[21] presents the current technology centered around acid hydrolysis of cellulose to sugar. Recent developments in cellulose pretreatment[18,21] show low pretreatment costs are attainable if the solvent can be recovered and recycled.

The current studies involved a series of chemical treatment combinations upon wheat straw (WS), and subsequent hydrolysis of released cellulose to fermentable sugars. Primary chemical treatment of WS was followed by additional, secondary treatment of residues with either acids or alkali. Following primary and secondary treatment the WS residues were hydrolyzed with cellulase, and final glucose yields were determined. Samples of residues and effluents taken after each reaction also were analyzed. Large-scale experiments for both saccharification and alcohol production were investigated in a glass-column reactor.

MATERIALS AND METHODS

Substrate

The straw used in the studies was from Arthur variety wheat. For convenience in handling in the laboratory, the straw was chopped into 2.5–7.5-cm lengths.

Primary Treatment

Ten WS samples of 10 g each were weighed into 1000-mL beakers, and 300 mL of 5% H_2SO_4 was added to each. A second set of ten samples was treated with 300 mL of 1% NaOH per sample (the straw was kept submerged with weights during the reaction period).

The acid and base reactions were done for two different lengths of time at two different temperatures. The H_2SO_4-treated samples were incubated 4 h with five of the ten samples at 25°C and five at 90°C. The NaOH-treated samples were incubated 1 h with five at 25°C and five at 90°C.

After incubation excess liquid was decanted, and the effluents were retained for hemicellulose analysis. WS residues were washed once and resubmerged in distilled water, and the pH of each was adjusted to 7 with either NaOH or H_2SO_4.

Secondary Treatment

After pH adjustment of the primary-treated WS, the beakers containing the samples were drained of their liquid, and one each of H_2SO_4- and NaOH-treated WS was further reacted with either ethylenediamine (EDA), NH_4OH, NH_3, or distilled water (control). Volume of liquid agent added to each sample was 200 mL. Concentrations of chemical agents were EDA, 28% and NH_4OH, 25%. For the anhydrous NH_3 reactions, acid- or base-treated WS samples were transferred to polyethylene bags, the bags evacuated of air, and each bag reinflated with approximately 8 g of NH_3 from a lecture bottle. After addition of the various chemical agents, all samples, both gas- and liquid-treated, were incubated 7 h at 25°C. Following incubation, the beakers containing liquid-treated WS samples were drained, and the damp, treated WS was transferred to plastic bags. All samples, including those treated with NH_3, were further incubated 3 days at 55°C.

After the 55°C incubation, treated materials were transferred from their plastic bags to beakers and each was washed once with 450 mL of distilled water. Fiber residues were resubmerged in distilled water, and pHs were adjusted to 7 with either H_2SO_4 or NaOH solution. The liquid was then drained from the WS residues, and the straw residues were dried to constant weight in a forced-air drying oven at 55°C. Subsequent experiments were conducted in similar fashion with pretreatment of straw with H_2SO_4 or NaOH at varying concentrations from 1 to 4 h at 25°C. After the samples were drained, the pretreated straw was treated with NH_4OH (20%) for 4 h at 55°C prior to enzymatic treatment.

Analytical Procedures

Lignin and total cellulose analyses were made by the methods of Goehring and Van Soest.[22] Free cellulose was determined by a method previously described,[19] namely enzymatically hydrolyzing the free cellulose of weighed samples and measuring the resultant glucose by high-pressure liquid chromatography (HPLC). Hydrolysis experiments were done with 200 mg of treated WS plus 10 IU cellulase per g of residue at 45°C for 6 h. HPLC analyses were run by passage through a 3.9-mm × 30-cm Waters carbohydrate analysis column (Waters Associates, Inc., Milford, MA) with acetonitrile–water (75:25) developing solvent. HPLC was used also for determining xylose (hemicellulose content) of effluents following chemical treatment of WS. Ethanol concentrations were determined by gas–liquid chromatography.

Column Reactor

Scale-up experiments for investigating the chemical–biological conversion of native wheat straw to alcohol were evaluated in a glass column reactor vessel. The pretreatments, saccharification, and fermentation were monitored in a 1.5-m column (11 cm i.d.). During the chemical/enzymatic steps, the column was sparged with air for maximal agitation. A heating coil was placed inside the column for circulating ethylene glycol for temperature control during both the modification and enzymatic procedures. The column was filled with 300–500 g of wheat straw and subjected to various chemical pretreatments prior to enzymatic saccharification and subsequent fermentation. Conversion rates of cellulose to glucose and production of ethanol are calculated based upon initial WS residue available in the column. A $\frac{1}{3}$-hp peristaltic pump was used to pump the chemical agents continuously through the residue bed in the column. *Saccharomyces uvarum* strain NRRLY-1347 was cultivated on YM medium in Fernbach flasks for 24 h yielding 10^8 cells/mL. One liter of the yeast cell inoculum was added to enzyme-hydrolyzed WS. A 24-h fermentation was run on this material and ethanol production determined.

RESULTS AND DISCUSSION

Chemical Pretreatment/Enzymatic Hydrolysis

The information presented in the initial phases of our research represent experiments conducted by the protocol depicted in Figure 1. Preliminary investigations[19] in our laboratory concerned primary chemical/biological modification of straw subsequent to enzymatic hydrolysis to sugar. Experiments described herein involve primary and secondary chemical modification steps prior to enzymatic saccharification and alcohol production.

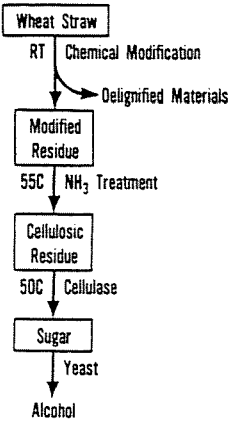

Fig. 1. Protocol for chemical modification experiments.

TABLE I
Primary and Secondary Pretreatments of Straw Followed by Enzymatic Saccharification

Chemical[a] pretreatment		Modified residual straw			Conversion[c] of cellulose to glucose (%)
		Percent loss[b]			
Primary	Secondary	Hemicellulose	Cellulose	Lignin	
5% H_2SO_4,	None	15	2	6	30
25°C for 4 h	EDA	51	5	69	98
	NH_4OH	40	1	25	55
5% H_2SO_4,	None	93	3	11	26
90°C for 4 h	EDA	93	10	75	59
	NH_4OH	92	7	19	30
1% NaOH,	None	32	13	17	51
25°C for 1 h	EDA	51	11	61	61
	NH_4OH	50	14	40	70
1% NaOH,	None	60	9	35	75
90°C for 1 h	EDA	60	17	47	72
	NH_4OH	60	9	29	83
Control (H_2O)		0	0	0	11

[a] As described in the Materials and Methods section.

[b] As determined by standard procedures for acid–detergent fiber analysis, cellulose, permanganate lignin, and neutral detergent for hemicellulose.[22]

[c] Hydrolysis of 200 mg of treated straw sample with 10 IU cellulase per g of residue. One IU = 1 μmol produced as glucose from filter paper/min. Conversion data were adjusted for weight gain from water addition to glucosyl moiety on hydrolysis. Formula: [(g glucose produced) (162/180) (100)]/(dry wt of cellulose, g) = percent conversion.

Table I depicts the enzymatic saccharification of wheat straw that has been pretreated with various chemical reagents. Pretreatment of WS with acid (5% H_2SO_4) or alkali (1% NaOH) resulted in little loss in the cellulosic component. Acid pretreatment at 90°C for 4 h resulted in only a 3% cellulose loss compared to 13% with alkali treatment. Acid pretreatment at room temperature (4 h) resulted in a 15% decrease in the hemicellulose fraction; at 90°C for 4 h, 93% was lost. Delignification was 11% or less in the acid pretreatments compared to 17–35% for alkali-treated samples.

At 25°C, the acid-pretreated WS treated with a fungal cellulase yielded a 30% conversion of the cellulosics to glucose, whereas the alkali-treated straw upon incubation with cellulase yielded 51% conversion.

Primary treatments of WS with H_2SO_4 or NaOH at 90°C for 4 h yielded cellulose conversions of 25 and 75%, respectively.

Primary/Secondary Treatment: Enzymatic Hydrolysis

Further experimentation centered around a secondary alkali treatment of acid–base-pretreated straw.

Secondary treatment of room temperature acid-pretreated straw with EDA yielded a 51 and 69% loss in hemicellulose and lignin, respectively. The highly corrosive EDA yielded an amorphous-appearing WS residue that, when treated with enzyme, yielded a 98% conversion as documented

in Table I. A similar result was obtained with the 90°C acid-treated straw and EDA; however, the glucose conversions were considerably reduced (59%). The production of furfural derivatives in the initial heat step may inhibit the enzymatic hydrolysis; this will be discussed later.

Secondary treatment with EDA of straw initially heated with alkali at room temperature yielded significant hemicellulose and lignin losses, 51 and 61%, respectively. Enzymatic conversion was 61%.

Secondary treatment with NH_4OH gave best results with the alkali-pretreated straw. Delignification was 40% with a 50% loss in hemicellulose with alkali straw pretreated at room temperature. Conversion rates to glucose were 70%. Straw pretreated with hot alkali and then treated further with NH_4OH yielded cellulose which was 83% convertible.

Primary/Secondary Treatment: Time Course

Since conversion percentages to glucose were highest with the alkali-pretreated straws, further evaluations were done by varying the alkali–acid concentrations and reaction time (1–4 h) in the pretreatments prior to secondary NH_4OH treatment. Figure 2 depicts the percent conversion of cellulose to sugar after 1–4 h of primary and 72 h of secondary chemical treatments prior to enzyme hydrolysis.

Maximal saccharification was obtained with the alkali-treated straws. Pretreatment with 0.55M NaOH for increasing periods of time yielded progressively higher percent saccharification, reaching 62% after 4 h. Pretreatment with 1% NaOH yielded conversion rates of 44–65% over 1–4 h. The highest percent conversion, 75%, was achieved with 2% NaOH for 4 h.

Acid pretreatments for 1–4 h with varying H_2SO_4 concentration (3–7%)

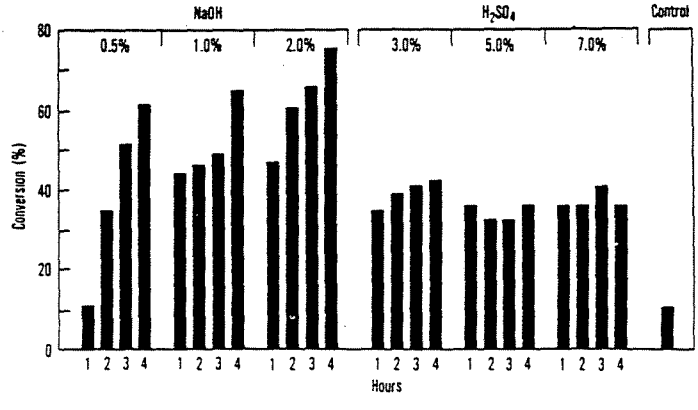

Fig. 2. Primary/secondary chemical pretreatment of straw prior to enzymatic saccharification. NaOH pretreatment: 0.5, 1.0, and 2.0% for 1–4 h prior to secondary treatment with NH_4OH at 55°C for 72 h. H_2SO_4 pretreatment: 3, 5, and 7% for 1–4 h prior to secondary treatment with NH_4OH at 55°C for 72 h.

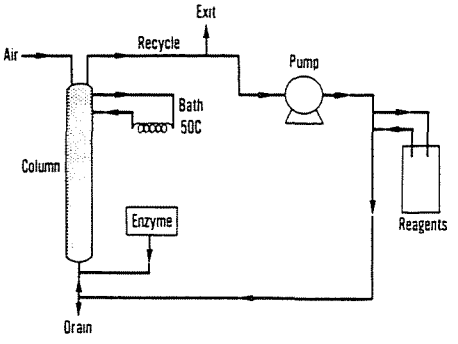

Fig. 3. Reactor vessel for straw saccharification and fermentation.

yielded approximately equal conversion rates, 32–41%. Control wheat straw treated with enzyme without prior chemical modification resulted in 11% conversion of the cellulose component to glucose.

As evidenced in Table I, extensive treatment with alkali resulted in exhaustive solubilization of hemicelluloses coupled to delignification. Cellulose component losses at room temperature were 14% or less with alkali treatment. Therefore, although the alkali pretreatment yields good saccharification, the hemicellulose component loss in this type of process must be taken into consideration. Since cellulosic residues contain from 10 to 35% hemicellulose, pretreatment or modification with chemicals may yield a carbon loss or solubilization and, therefore, must be calculated in terms of efficiency of total sugar conversion.

Batch Column Reactor Vessel for Saccharification and Alcohol Fermentation

Figure 3 depicts a scale-up unit used for hydrolysis for wheat straw similar to the small-scale experiments. The column reactor was used for experiments with 300–500 g of straw. Various treatments were considered in the scale-up studies with a look toward optimization of the chemical modification and enzymatic saccharification steps. Chemical reagents were pumped continuously through the temperature-controlled column with aeration. The modified WS was H_2O washed with 3–4 volumes before addition of buffered enzyme at 50°C.

Table II contains data from several experiments on sugar formation and alcohol production from chemically modified straw in the reactor column. Pretreatment of straw with NaOH, which appeared to be optimum for enzymatic hydrolysis, was accompanied by a considerable loss in straw dry weight, i.e., 18–25%. As a step toward practical processing, the alkali-treated straw was neutralized with acid or water prior to enzymatic hydrolysis. Neutralization was quite satisfactory by washing with H_2O. The nature of the products formed during alkaline treatment of

TABLE II
Glucose and Alcohol Production from Chemically Modified and Enzymatically Treated Straw in Column Reactor

Experiments Chemical pretreatments[a]	Straw (g)/ H_2O volume (L)	Total sugar produced[b] Glucose (g)	Xylose (g)	Conversions[c,d] Cellulose to glucose (%)	Glucose to ethanol (%)
NaOH (1%) 24 h	300 g/8 L	35	13.5	26	42
NaOH (0.5%) 6 h	300 g/6 L	39	23.9	30	41
NaOH (0.5%) 24 h	500 g/4 L	40	16.4	18	30
NH_4OH (10%) 24 h	300 g/4 L	20	5.2	15	31
Anhydrous NH_3 (6 g) 6 h	100 g/L	20	7.0	15	33

[a] Chemical modification experiments conducted at room temperature.
[b] Enzymatic saccharification conducted on the resultant modified residue with 10 IU/mg of residue at 45°C for 6 h.
[c] Same as c in Table I.
[d] Represents alcohol produced from the saccharified glucose available compared to theoretical yield of 2 mol ethanol from 1 mol glucose.

straw and H_2O wash are unknown. Recent experiments reported the formation of antimicrobial substances during heat treatment of xylose with alkali.[23] Such materials could retard fermentation and the separation of alkali-treatment liquor would be indispensable.

A large percent of the dry weight lost in alkali treatment is the hemicellulose component, which is 25% of wheat straw. In the column reactor, hemicellulose losses of 50–60% were observed in the alkali-treated straws.

In experiments conducted with 300–500 g or more of straw, reacted with 0.5% $NaOH/NH_4OH$ for 6 h, biomass losses were 15–20%.

This hemicellulose fraction obtained by alkali treatment could be neutralized and precipitated with ethanol for use as carbon source for xylose-fermenting organisms.

In the various trials, conversion of the cellulosic components of wheat straw to fermentable sugar ranged between 15 and 30%. The crude fungal cellulase–hemicellulase preparations used to treat the modified straw yielded also a substantial quantity of the fermentable C-5 sugar, xylose. From 300 g of native WS, the yield of xylose from enzyme-treated, modified straws was 5–24 g. This xylose fraction is exclusive of the hemicellulose fraction lost in the aqueous extraction phase of the alkali-treated straws.

Ethanol yields from the saccharified sugars in the column inoculated with an *S. uvarum* strain NRRLY-1347 at 10^8 cells/mL were 30–42% of theoretical. Possibly, yields were low because inhibition substances were generated during the chemical treatments or enzymatic saccharification and because the sugar concentration was only 1–3% in the column reactor.

It would be of considerable advantage to precipitate the extracted hemicelluloses with the modified cellulosic residues and then to follow with an enzymatic saccharification to yield both C-6 and C-5 sugars for fermentation.

References

1. W. E. Tyner, *Biotechnical. Bioeng.* (to appear).
2. Y. W. Han, *Appl. Microbiol.*, **29,** 510 (1975).
3. Y. W. Han and A. W. Anderson, *Appl. Microbiol.*, **30,** 930 (1975).
4. L. A. Lindenfelser, R. W. Detroy, J. M. Ramstack, and K. A. Worden, *Dev. Ind. Microbiol.*, **20,** 541 (1979).
5. F. Rexen and K. V. Thomsen, *Anim. Feed Sci. Technol.*, **1,** 73 (1976).
6. W. N. Garrett, H. G. Walker, Jr., G. O. Kohler, A. C. Waiss, Jr., R. Graham, N. E. West, and M. R. Hart, *Proc. Am. Assoc. Anim. Sci.*, **25,** 317 (1974).
7. H. Tarkow and W. C. Feist, *Adv. Chem. Ser.*, **95,** 197 (1969).
8. P. J. Van Soest, *Fed. Proc.*, **32,** 1804 (1973).
9. M. R. Hart, R. P. Graham, P. F. Hanni, W. C. Rockwell, M. G. Walker, Jr., G. O. Kohler, A. C. Waiss, Jr., and W. N. Garrett, *Feed Stuffs*, **47,** 24 (1975).
10. E. Beckman, *Chem. Abstr.*, **16,** 765 (1921).
11. A. C. Waiss, Jr., J. Guggolz, G. O. Kohler, H. G. Walker, Jr., and W. N. Harrett, *J. Anim. Sci.*, **35,** 109 (1972).
12. E. J. del Rosario, A. L. Gonzales, L. C. Vilela, S. A. Capulso, C. R. Pontiveros, A. R. Torillo, A. T. deOcampo, and R. D. Alolod, *Philippine J. Crop Sci.*, **2,** 1 (1977).
13. C. J. Israilides, G. A. Grant, and Y. W. Han, *Appl. Environ. Microbiol.*, **36,** 43 (1978).
14. Y. W. Han and C. D. Callihan, *Appl. Microbiol.*, **27,** 159 (1974).
15. G. C. Fahey, Jr., S. S. Masters, and B. L. Miller, *Ill. Res.*, **20,** 10 (1978).
16. G. A. Grant, Y. W. Han, A. W. Anderson, and K. L. Frey, Technical Paper No. 4289, Agricultural Experiment Station, Oregon State University, Chap. 50, p. 599.
17. J. W. Dunning and E. C. Lathrop, *Ind. Eng. Chem.*, **37,** 24 (1945).
18. M. R. Ladisch, C. M. Ladisch, and G. T. Tsao, *Science*, **201,** 743 (1978).
19. R. W. Detroy, L. A. Lindenfelser, G. St. Julian, Jr., and W. L. Orton, *Biotechnol. Bioeng. Symp.*, **10,** 135 (1980).
20. Y. W. Han, *Adv. Appl. Microbiol.*, **23,** 119 (1978).
21. H. F. Wenzel, *The Chemical Technology of Wood* (Academic, New York, 1970), p. 157.
22. H. K. Goering and P. J. Van Soest, *U.S. Dept. Agric. Handb.*, 379 (1970).
23. N. Kato and I. Shibasaki, *J. Ferment. Technol.*, **52,** 177 (1974).

Accepted for Publication December 3, 1980